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Status of Long-Term Asymptomatic HIV-1 Infection Correlates With Viral Load but not With Virus Replication Properties and Cell Tropism

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Qualitative and quantitative virological parameters were investigated in 68 long-term nonprogressor (LTNP) HIV-1-infected patients and 9 slow-progressor controls. LTNP status was defined as an asymptomatic HIV infection for at least 8 years, a stability of $CD4^+$ cell counts $\geq$600 cells/mm^3 and no antiretroviral therapy. LTNP subjects exhibited a lower median plasma RNA load than controls (6,000 vs 40,000 RNA copies/ml) despite a wide range of values in both groups. When compared to the control group, LTNP subjects also exhibited a lower virus isolation rate (65% vs 100%) and cell-associated viremia (0.75 vs. 56.8 number of infectious unit/million cells) when CD8-depleted $CD4^+$ cells were tested. By contrast, no major differences in virus replication properties or cell tropism were observed. After 1 year of follow-up, no major overall changes in the virological parameters was observed in the 50 LTNP subjects evaluated at this time. However, nine patients had started antiretroviral therapy, and six others had increased viral loads. Despite the progression observed during the first year of follow-up, the hypothesis that there is a specific subgroup of LTNP patients who will not develop disease cannot be ruled out as yet. ***J. Med. Virol. 58:256–263, 1999.***

KEY WORDS: long-term non-progressor; viral load; heterogeneity; replication properties; progression

INTRODUCTION

Following primary infection with human immunodeficiency virus type 1 (HIV-1), the majority of patients experience an asymptomatic period after seroconversion and before the development of AIDs [Pantaleo et al., 1993]. The duration of this period of clinical latency is highly variable among the infected individuals. The majority of patients develop AIDS within approximately 9 to 10 years after HIV infection. However, 5–10% of all HIV-1-infected individuals remain clinically healthy and immunologically normal despite prolonged HIV infection [Learmont et al., 1992; Sheppard et al., 1993; Buchbinder et al., 1994; Easterbrook, 1994; Schrager et al., 1994]. These patients, generally termed long-term nonprogressors (LTNPs), are usually defined as being infected over a decade, lacking clinical symptoms, and maintaining normal and stable $CD4^+$ T-cell counts. It is still unclear whether these patients constitute a distinctive subgroup that will not develop disease or only correspond to the casual finding of some rare individuals with slower progression to AIDS.

During the past three years, several studies have been conducted to understand the basis for nonprogression of the disease and have suggested that persistent low-level viral replication was controlled more efficiently in nonprogressor patients than in progressors

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[Schwartz et al., 1994; Cao et al., 1995; Pantaleo et al., 1995; Rinaldo et al., 1995; Clerici et al., 1996; McKenzie et al., 1996]. Multiple host and viral factors may contribute to this virus containment. Host factors may result in differential susceptibility of the host cells to viral infection and its pathogenic effects or in the development of strong cellular and/or humoral immune responses [Barker et al., 1995; Cao et al., 1995; Rinald et al., 1995; Montefiori et al., 1996]. The viral factors may include particular susceptibility to virus-inhibitory $CD8^+$ cell responses and/or to neutralizing antibodies. Other studies suggested that nonprogression in some patients may be the result of infection with attenuated viruses [Deacon et al., 1995; Iversen et al., 1995; Kirschhoff et al., 1995].

It still remains unclear what are the relative contributions of host factors and viral factors in determining the lack of progression of HIV disease in some individuals. It could be hypothesized that nonprogression to AIDS depends on a fine balance among multiple and complex interactions between host and virus in the same individual. However, despite this complexity, the study of these subjects may yield important information on the determinants of nonprogression and, consequently, may increase understanding of the general physiopathology of HIV infection. This information may also be useful for defining prognosis, as well as new therapeutic or vaccine strategies. Such a study would require a large number of nonprogressor subjects and the inclusion of relevant control subjects.

With the goal of characterising the parameters that contribute to nonprogression to AIDS, a French cohort of LTNP patients, also designated as ALT (long-term asymptomatic) cohort, was constituted. Virological and immunological studies will be undertaken on the LTNP patients over 5 years. In this study, we present preliminary qualitative and quantitative virological analyses of LTNP subjects at entry and other 1 year of follow-up.

PATIENTS AND METHODS

Patients

LTNP status was defined as asymptomatic HIV-1 infection for at least 8 years with stable $CD4^+$ cell counts and no antiretroviral therapy. Inclusion criteria were a $CD4^+$ cell count ≥ 600 cells/mm^3, which had not decreased during the last 5 years. Patients in the control group had asymptomatic HIV infection for 8 years with $CD4^+$ cell counts between 300 and 400 cells/mm^3, a CD4 decrease of at least 25% in the last 3 years, and no antiretroviral therapy.

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood as described previously [Barre-Sinoussi et al., 1983]. CD8-depletion was carried out using immunomagnetic beads (Dynal, Oslo, Norway).

Plasma Virus Quantitation

HIV-1 RNA was quantified by using nucleic acid sequence-based amplification assay (NASBA HIV-1 RNA QT, Organon Teknika, Boxtel, The Netherlands) according to the instructions of the manufacturer. The lower limit of detection of this assay was 800 RNA copies/ml of plasma. For the samples yielding values below 800 RNA copies/ml, the Amplicor HIV Monitor assay (Roche Diagnostic Systems Inc., Neuilly/Seine, France) was used in combination with the RNA extraction procedure of the NASBA assay, with a detection threshold of 20 RNA copies/ml.

Virus Isolation and Detection

Virus isolation was attempted from plasma, peripheral blood mononuclear cells (PBMCs), and CD8-depleted lymphocytes ($CD4^+$ cells) using conventional coculture assays [Barré-Sinoussi et al., 1983]. Virus yields were quantified in culture supernatants by p24 immunoassay (NEN Research Products, Du Pont de Nemours, Les Ulis, France). Virus stocks were obtained after just one passage in PBMC cultures and used for further evaluation.

CD4-cell-associated viremia was determined from cocultures of 4×10^6 CD8-depleted $CD4^+$ cells using the procedure recommended by the French National Agency for AIDS Research [Rouzioux et al., 1992]. HIV-1 titers were expressed as the number of infectious units per million of $CD4^+$ cells (NIU/10^6 $CD4^+$ cells).

HIV-1 DNA was detected directly from PBMCs by PCR using both a validated home-made assay with primers located in the *pol* gene [Candotti et al., 1991] and the Amplicor HIV-1 test (Roche Diagnostic Systems, Inc. Branchburg, USA). As a control, the β-globin gene was amplified successfully from all DNA samples studied. Each PCR assay was performed with 1 μg of PBMC DNA. The detection threshold was 33 HIV-1 DNA copies/10^6 cells.

Characterization of Virus Replication Properties and Cell Tropism

Monocyte-derived macrophages (MDM) were obtained from fresh PBMCs from healthy donors as previously described [Mabondzo et al., 1991]. MDMs were infected with 50 $TCID_{50}$ of virus and HIV replication was detected by measuring the reverse transcriptase (RT) activity in the culture supernatants [Schwartz et al., 1988].

The same methods were used with MT-2 and CEM clone 13 T-cell lines. Syncytium-inducing (SI) and nonsyncytium-inducing (NSI) virus phenotypes were determined in MT-2 cells as previously described [Tamalet et al., 1994].

The recombinant cell lines HeLa-CD4 LTR LacZ (clone P4-2) and HeLa-CCR-5-GFP (clone P4-C5) were a gift from F. Arenzana-Seisdedos. The HeLa-CD4 LTR LacZ cells, which constitutively express the CXCR-4 molecule, were transduced with human CD4 and carry an integrated *Escherichia coli* β-galactosidase reporter gene driven by a HIV-1 LTR [Amara et al., 1997]. HeLa-CCR-5-GFP cells were derived from the HeLa-CD4 LTR LacZ cells cotransfected with the CCR-5-GFP

TABLE I. Virological Characteristics of LTNP Subjects and Controls at the First Blood Sample Evaluation

	LTNPs $n = 68$	Controls $n = 9$	P[a]
Plasma viral load (RNA copies/ml plasma)			
Median	6,000	40,000	.040[b]
Range	<20–860,000	2,200–1,860,000	
$CD4^+$ cell virus titer (NIU/million $CD4^+$ cells)			
Median	0.75	56.85	.006[b]
Range	<0.1–>875.1	0.4–>875.1	
No. of virus isolations from plasma	9/53	2/9	.650
(% of tested)	(17)	(23)	
No. of virus isolations from PBMC	21/49	5/8	.272
(% of tested)	(43)	(63)	
No. of virus isolations from $CD4^+$ cells	34/52	9/9	.025[b]
(% of tested)	(65)	(100)	
No. of HIV-1 DNA detection in PBMC	58/68	9/9	.504
(% of tested)	(85)	(100)	

[a]Statistical comparison of LTNP subjects versus controls was tested by Mann-Whitney and Fisher's Exact tests as appropriate.
[b]Significant result.

vector [Amara et al., 1997]. HIV replication was detected by measuring the β-galactosidase activity.

The HIV-1 laboratory strains HIV-1_{LAI} (LAI), HIV-1_{MN} (MN) and HIV-1_{BaL} (BaL) were included in the study as internal reference strains and were propagated serially in PBMC cultures [Barré-Sinoussi et al., 1983; Gartner et al., 1986; Gurgo et al., 1988].

Statistical Analysis

All analyses were carried out using the SPSS software. Categorical variables were compared using Fisher's exact test and, for continuous variables, the nonparametric Man-Whitney test. Spearman rank correlation test was used to study the association of continuous variables. All the values shown were derived from the results of a two-tailed test. $P < .05$ was considered statistically significant.

RESULTS

LTNP Cohort and Control Enrollment

Currently, 68 patients fulfilling the inclusion criteria have been enrolled in the LTNP cohort. Due to the increasing frequency of therapy among subjects with $CD4^+ < 500$ cells/mm^3, the enrollment of asymptomatic control subjects with no anti-HIV therapy was more difficult. Therefore only nine control patients had been enrolled in the study at the end of the recruitment period, and the inclusion of additional control subjects did not seem to be possible any more. The median $CD4^+$ T cell counts were 838 cells/mm^3 (range: 625–1879 cells/mm^3) as measured in the various referring clinical centres. The control group had a median $CD4^+$ cell count of 350 cells/mm^3 (range: 276–354 cells/mm^3).

Virus Quantitation

As shown in Table I, the levels of plasma HIV-1 RNA copies detected in the 68 LTNP subjects were significantly lower than those detected in the 9 controls ($P = .04$). Median plasma virus load was 6,000 HIV-1 RNA copies/ml of plasma in the LTNP subjects compared to 40,000 RNA copies/ml of plasma in the controls. A wide range of plasma viral loads was observed among the LTNPs with HIV-1 RNA levels ranging from <20 up to 860,000 RNA copies/ml plasma and a similar range was observed for the controls (Table I). A total of 21 LTNPs had HIV-1 RNA levels below the value of 800 RNA copies/ml plasma, which was the limit of detection of the NASBA assay. Among them, 12 had RNA levels below 200 copies/ml and 2 had no detectable RNA, using the Roche-modified assay with a threshold of 20 copies/ml. Among the 47 LTNPs with plasma viral load higher than 800 copies/ml, 30 had a viral load higher than 10,000 copies/ml and 3 had a viral load higher than 500,000 copies/ml despite fulfilling the inclusion criteria.

Infectious HIV-1 was quantified in the $CD4^+$ cells of 59 LTNP subjects and 7 controls following $CD8^+$ cell depletion. Median $CD4^+$ cell infectious titre was 0.75 number of infectious unit (NIU) per million $CD4^+$ cells, with values ranging from 0.1 up to 875.1 NIU/million $CD4^+$ (Table I), the lower and upper limits of detection of our assay. Sixteen LTNPs exhibited infectious titres below the limit of detection of the assay (<0.1 NIU/million $CD4^+$ cells). In controls, the median $CD4^+$ cell infectious titre was significantly higher than in the LTNPs (56.85 NIU/million $CD4^+$ cells, $P = .0057$) with a range similar to that observed in the LTNPs.

Virus Isolation and Viral DNA Detection

HIV-1 was isolated more frequently from PBMCs and $CD4^+$ cells than from plasma. The rate of virus isolation from $CD4^+$ cells was significantly higher for LTNP subjects ($P = .025$) while the rates of isolation from PBMC and plasma did not differ significantly between both groups (Table I). The kinetics of HIV-1 growth during primary isolation were highly heterogeneous both in the LTNP cohort and the controls. The median day for the first detection of virus in culture occurred significantly later for LTNPs than controls

TABLE II. Comparison of Replication Properties of LTNP Isolates Versus Control Isolates

	LTNPs	Controls	P^a
Kinetics of replication[b] (days) on:			
PBMC [median (range)]	13 (6–30)	9 (6–18)	0.008
MDM [median (range)]	13 (6–19)	9 (9–15)	0.264
Replication on continuous cell lines[c] [no. of replicating isolates (% tested)]:			
MT-2	2/37 (5)	3/9 (33)	0.062
CEM clone 13	0/31 (0)	0/6 (0)	NA
Replication on recombinant cell lines[d] (no. of replicating isolates (% tested)]:			
HeLa-CCR-5-GFP clone P4-C5	37/37 (100)	9/9 (100)	NA
HeLa-CD4 clone P4-2	0/37 (0)	1/9 (14)	0.438

NA, nonapplicable.
[a]Statistical comparison of LTNP isolates versus controls was tested by Mann-Whitney and Fisher's Exact tests as appropriate.
[b]Day of RT activity peak measured in the culture supernatants.
[c]Virus replication was detected by RT activity detection in the supernatants.
[d]Virus replication was detected by p24 production and in situ β-galactosidase activity detection.

only in the case of isolation from $CD4^+$ cells (14 and 7 days, respectively, $P = .01$). No difference between LTNPs and controls was observed with respect to the levels of p24 production in culture supernatants (data not shown). HIV-1 DNA was detected in 58 LTNPs among the 68 tested. Surprisingly, it was not detected in 10 LTNPs (15%) using both the Amplicor HIV-1 test with a threshold of 33 DNA copies/10^6 cells and different nested PCR assays with various primer combinations. In the 10 cases, there was a clear serological evidence of HIV-1 infection and the HIV-1 subtype implicated was subtype B as determined by a specific serological assay kindly undertaken by F. Barin as reported previously [Barin et al., 1996].

In Vitro Replication Properties of the Virus Isolates

The kinetics of replication from 37 LTNP virus isolates and 9 control isolates was studied in activated PBMC from HIV seronegative donors by measuring RT activity in culture supernatants after infection with a standardized 50 $TCID_{50}$ inoculum of each virus. Six LTNP isolates and four controls showed a kinetics of replication similar to that of the two laboratory strains LAI and MN taken as references. A high peak of RT activity (>100,000 cpm/50 μl) was observed 6 days after infection, and those isolates were classified as rapid/high (R/H) viruses. By contrast, nine LTNP isolates exhibited a very low rate of replication with a low peak of RT activity (<40,000 cpm/50 μl) obtained 23 to 30 days after infection and were classified as slow/low (S/L) isolates. The remaining 22 LTNP isolates and 5 controls exhibited intermediate (I) replication rates. Overall analysis showed that the peak of RT activity in PBMCs was obtained significantly later for LTNP isolates than for controls ($P = .008$) (Table II). Virus growth in monocyte-derived macrophages (MDM) was investigated for 27 of the 37 LTNP isolates (5 R/H, 6 S/L and 16 I) and for 5 of the 9 controls (4 R/H and 1 I) using the strain $HIV\text{-}1_{BaL}$ (BaL) as a reference MDM-tropic strain. Again, LTNP isolates and control isolates exhibited diverse kinetics profiles as compared to BaL but no significant difference was observed between LTNP isolates and controls in MDM cultures (Table II).

The ability of cohort isolates to replicate in the T-cell lines MT-2 and CEM were then investigated. Two out of 37 LTNP isolates and three out of the nine control isolates replicated on MT-2 cells exhibiting a syncytium-inducing (SI) phenotype. All the other isolates were considered to have a non-syncytium inducing (NSI) phenotype. None of 6 controls (2 SI and 4 NSI) and 31 LTNP isolates (1 SI and 30 NSI) tested was capable of infecting CEM clone 13 cells. All SI isolates did infect MDM and should be considered rather as dual-tropic viruses than real T-tropic viruses.

The ability of these isolates to replicate on recombinant cell lines expressing CCR-5 and/or CXCR-4 was investigated (Table II). Target cells were the recombinant HeLa-CD4 clone P4-2 expressing CD4 and CXCR-4, and the HeLa-CCR-5-GFP clone P4-C5 expressing CD4, CXCR-4 and CCR-5 receptors. HeLa-CCR-5-GFP clone P4-C5 cells were infected by all the 37 LTNP and 9 control isolates tested. HIV-1 laboratory strains LAI, MN and BaL, used as controls, showed a high level of replication in HeLa-CCR-5-GFP clone P4-C5 while only LAI and MN grew on HeLa-CD4 clone P4-2. All the LTNP and control isolates but one failed to infect HeLa-CD4 clone P4-2 (Table II). These results indicated that all but one primary isolate tested in this study were CCR5-dependent. The only isolate that infected productively HeLa-CD4 clone P4-2 cells exhibited a SI phenotype in MT-2 cells.

Relationship Between Virological Parameters in LTNP Subjects

As shown in Figure 1A, plasma RNA load correlated significantly with CD4 cell-associated viremia ($P < .0001$). As expected, plasma RNA load was significantly higher for LTNP subjects with HIV isolation from

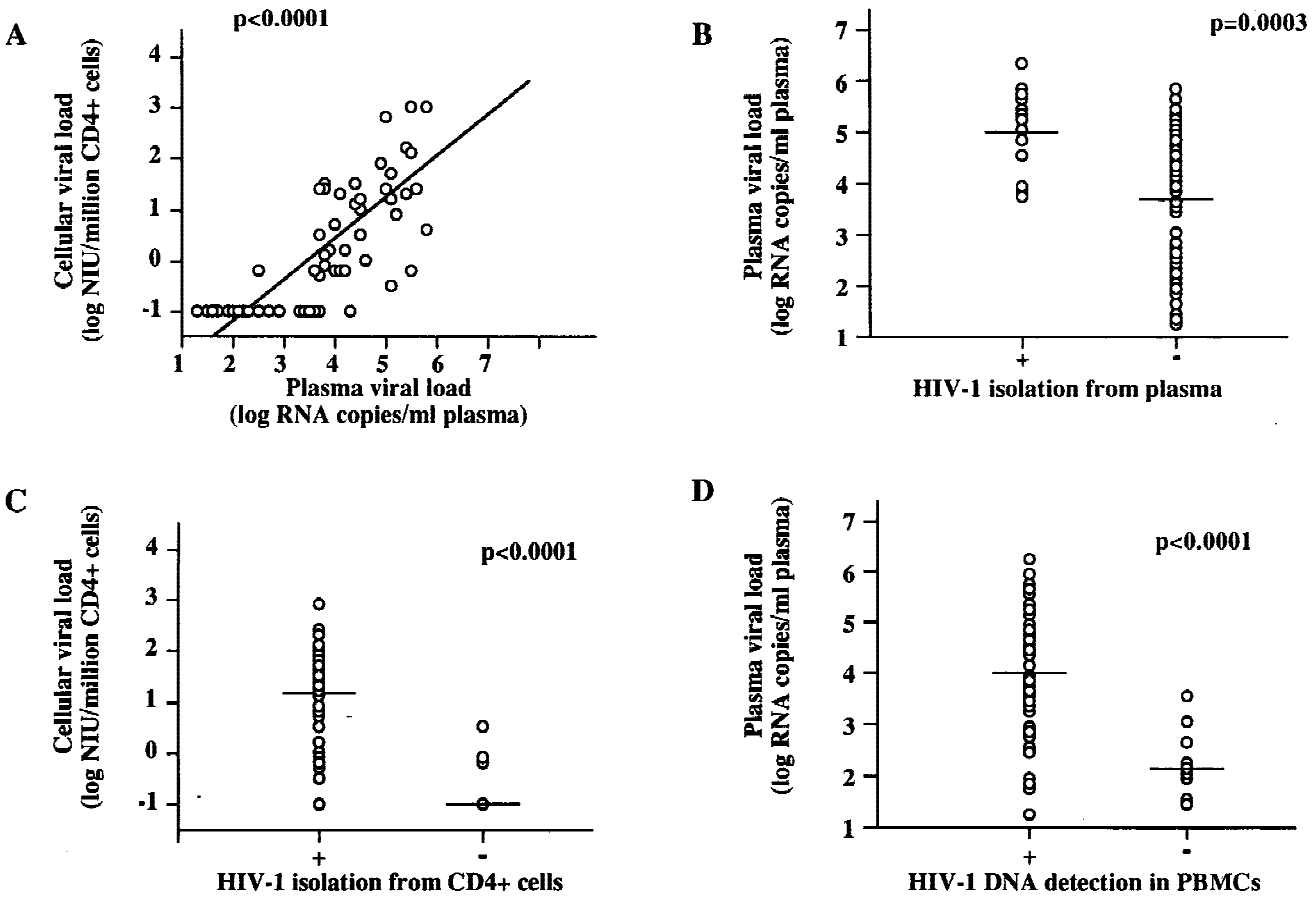

Fig. 1. Relationship between virological parameters in LTNP subjects. Plasma viral loads were compared with cellular viral loads **(A),** HIV-1 isolation from plasma **(B)** and HIV-1 DNA detection in PBMCs **(D).** Cellular viral loads were compared with HIV-1 isolation from $CD4^+$ cells **(C).** The horizontal bars in panels B, C, and D denote median values.

plasma than for those with no isolation ($P = .0003$) (Fig. 1B). Accordingly, CD4 cell-associated viremia was higher in the case of HIV isolation from CD4 cells than in the case with no isolation ($P < .0001$) (Fig. 1C). The delay for the positivity of p24 antigen in culture supernatant during isolation assay was inversely correlated with plasma virus load and cell-associated viremia, respectively. The detection of HIV DNA in PBMC was significantly associated with a higher plasma RNA load (Fig. 1D). Median plasma RNA load was 11,400 RNA copies/ml in subjects with detectable HIV DNA and 118 RNA copies/ml in subjects with undetectable DNA. A correlation was also observed between the absence of DNA detection in peripheral blood and the absence of detectable cell-associated viremia (data not shown). Overall these results suggested that both the rate of virus isolation and HIV DNA detection were highly associated with virus load. However, virus RNA and DNA might be detected in the peripheral blood compartment whereas attempts to isolate infectious virus from plasma or cells of the same sample failed, demonstrating the lack of absolute correlation between the physical presence of the virus and its infectivity. Surprisingly, no significant relationship was evidenced between CD4 cell counts and virological parameters (data not shown).

Virological Evaluation After One Year of Follow-up

Due to the high plasma viral loads observed at entry in some LTNP patients, antiretroviral therapy was started for three of them and, consequently these treated patients were withdrawn from LTNP cohort. Among the remaining LTNP subjects, a second virological evaluation was completed for 50 subjects at 1 year after the first one. No significant variation of RNA plasma loads was observed after 1 year. Median RNA plasma loads were 5,200 and 7,200 RNA copies/ml for the first and second evaluation, respectively. However, six LTNP patients exhibited a significant increase ($\geq$0.5 log) of their HIV RNA level in plasma (Fig. 2). At entry, three of them had exhibited RNA plasma loads below 200 RNA copies/ml and the other three had had RNA plasma loads between 201 and 10,000 RNA copies/ml. None of the 21 LTNP subjects with more than 10,000 HIV RNA copies/ml at entry showed a significant increase of their RNA plasma load 1 year later. After this second evaluation, antiretroviral therapy

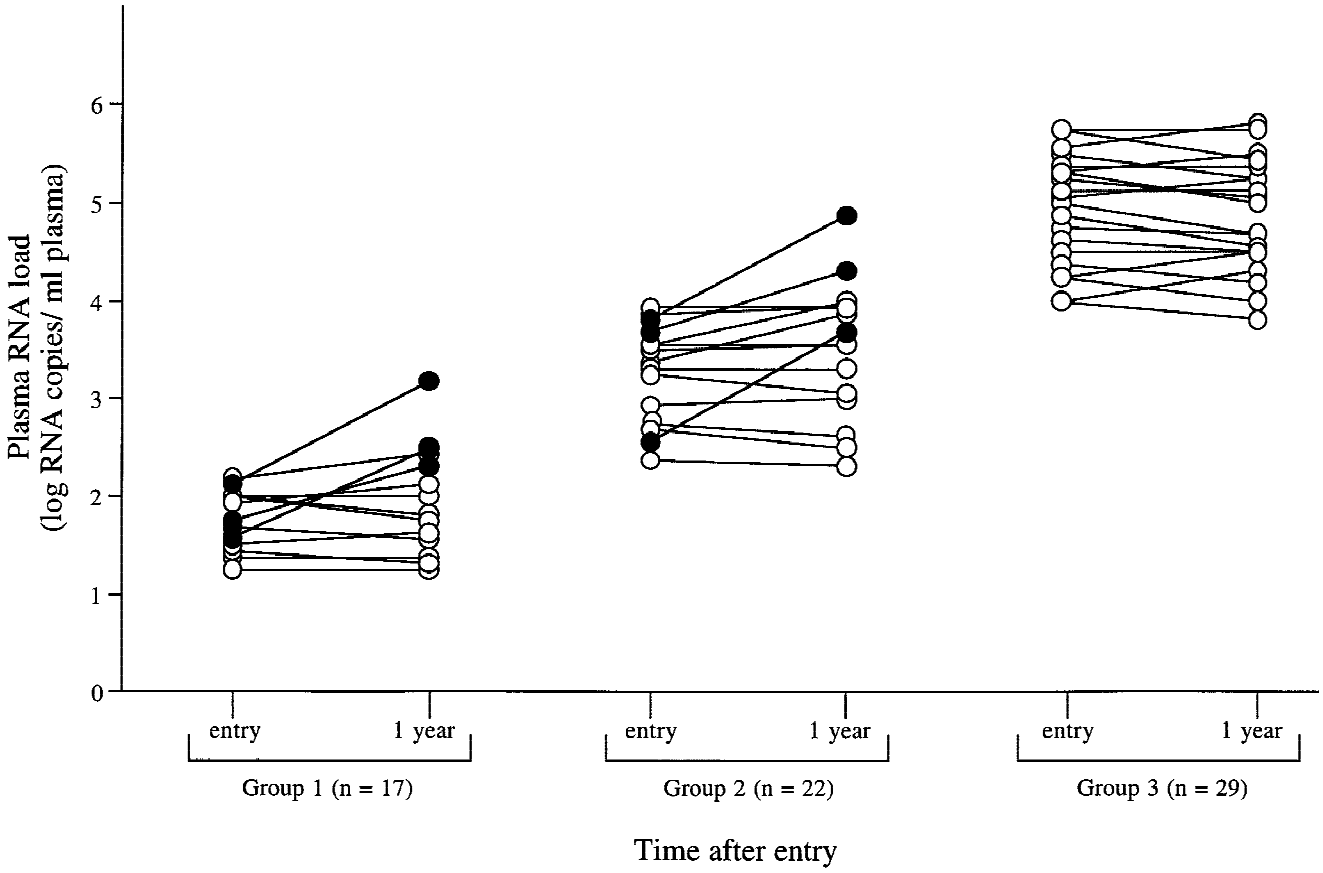

Fig. 2. Variation of plasma RNA loads after 1 year of follow-up. A second virological evaluation was completed for the LTNP subjects at 1 year after the first one. For reasons of clarity, the subjects have been separated into three groups according to their plasma RNA load at entry: 200 copies/ml or less (group 1), 201 to 10,000 copies/ml (group 2) and more than 10,000 copies/ml (group 3). *Filled circles* denote subjects showing a significant increase (≥0.5 log) of their plasma RNA loads, and *open circles* denote subjects showing no significant (<0.5 log) variation of their plasma RNA loads.

was started for six LTNP subjects with stable but high RNA plasma loads (>10,000 copies/ml). No significant variation of $CD4^+$ cell count was observed within the LTNP group as a whole during the first year of investigation, but two subjects with high viral load exhibited a significant decrease of $CD4^+$ cells during this period (data not shown).

DISCUSSION

The aim of the study was to characterise the virological parameters of patients who have experienced neither clinical nor immunological progression of HIV disease despite prolonged periods of HIV infection. The study sought appropriate criteria for characterising HIV-infected long-term nonprogressors. The observations regarding nonprogression have been reported to be highly dependent on the definition of this status and the duration of follow-up [Salhi and Costagliola, 1997]. In our study, LTNP status was defined as a HIV-1 infection for at least 8 years with no antiretroviral therapy and stable $CD4^+$ cell counts. Although the absolute $CD4^+$ cell count has been recognised to be a strong marker of progression to AIDS, it was less clear whether this marker was a useful indicator of nonprogression [Strathdee et al., 1996]. Most studies have included in their definition a criterion based on $CD4^+$ counts [Salhi and Costagliola, 1997], but it has been reported that $CD4^+$ lymphocyte levels varied widely within and between individuals, and might fluctuate depending on external factors [Raboud et al., 1995]. A strict definition was applied based on a $CD4^+$ count above 600 cells/mm^3 with stability of the $CD4^+$ slope over 5 years. Moreover, subjects taken as controls in the study were slow progressors with asymptomatic HIV infection. Consequently, two populations of HIV-1-infected subjects exhibiting minimal differences concerning the stage of infection were compared.

HIV-1 load in infected individuals has been reported to have considerable physiopathological and clinical significance, since HIV DNA copy numbers in PBMCs as well as the cell-free viral load in plasma correlated with the rate of subsequent disease progression and time to AIDS [Hogervorst et al., 1995; Mellors et al., 1996; Vesanen et al., 1996]. LTNP subjects exhibited lower plasma loads and $CD4^+$ cell-associated viraemia than the control subjects with slow progressive disease. However, virus loads were found to vary widely among these LTNP subjects. A majority of LTNP subjects ex-

hibited low, even undetectable, viral loads as reported previously. Partial data from the 1-year follow-up indicated that plasma virus loads had remained stable in the majority of the LTNP subjects. All these results suggest a strong control of virus replication in these subjects. The mechanisms used by these subjects to control virus replication over time still remain unclear, but their cellular and humoral immune responses, as well as the genetic characteristics of the corresponding viruses, are still under investigation. In contrast to the general profile of nonprogressor subjects, the combination of stable $CD4^+$ cell counts and high virus loads (>10,000 RNA copies/ml) was observed in 44% of the subjects of our cohort. Thus, a high level of virus replication cannot be considered as a fully discriminant feature of rapid progression to clinical disease as reported by others [Rump et al., 1996], and the lack of disease progression in the LTNP subjects might involve other significant physiopathological components [Zinkernagel and Hengartner, 1994; Rump et al., 1996; Vesanen et al., 1996]. In addition, the one-year follow up of the cohort showed that a minority of the LTNP patients (6 of 50 in our study) experienced a significant increase of their plasma virus loads, strongly suggesting a progression of HIV infection. This indicated that the control of viral replication over time was not a property common to all the individuals defined as LTNP subjects. Overall results confirmed that plasma virus load remained a significant major parameter to characterise the infection profile in terms of stability or evolution within the LTNP group.

The prevalence of virus isolation from the $CD4^+$ cells was significantly lower in the LTNP patients than in patients with slow progressive infection as expected from the results of $CD4^+$ cell-associated viremia. No difference between the two groups of patients was observed in virus isolation from plasma and PBMCs. The increased rate of isolation from purified $CD4^+$ cells suggested that $CD8^+$ cell depletion enhanced more efficiently virus isolation for the LTNP patients than for the asymptomatic controls. Additional studies are still in progress in order to investigate if the LTNP $CD8^+$ cells had a stronger ability to control HIV-1 infection in culture than the $CD8^+$ cells of the control subjects. The absence of virus isolation from some LTNP subjects was consistent with low plasma and cellular virus loads. Further studies are required to establish whether qualitative genetic virus defects might account for such failure of isolation as suggested by others [Deacon et al., 1995; Iversen et al., 1995; Kirschhoff et al., 1995]. However, HIV-1 isolates were recovered from the majority of the LTNP patient blood samples, indicating that the majority of such individuals harbored infectious viruses that were competent for replication. As a general trait, in vitro culture experiments revealed that LTNP isolates had a slow/low phenotype. It should be noted that some LTNP isolates exhibited a rapid/high phenotype.

All LTNP isolates replicated in MDM, and all but two were NSI. A similar NSI phenotype was observed for the majority of the control isolates. However, two LTNP isolates replicated as efficiently in MDMs as in the MT-2 cell-line where they exhibited SI phenotype. To date, one of these two LTNP patients experienced a disease progression and started antiretroviral therapy 1 year after the first virological evaluation, suggesting that the presence of SI viruses might predict progression as reported for other HIV-infected subjects [Schuitemaker et al., 1992]. The C-X-C chemokine receptor CXCR-4, and the C-C chemokine receptors CCR-5 and CCR-2b have been identified as the principal entry coreceptors for SI and NSI HIV-1 strains, respectively [Dragic et al., 1996; Oberlin et al., 1996]. Using HeLa-CD4 and HeLa-CCR-5-GFP recombinant cell lines, we confirmed that infection with the NSI isolates from LTNP subjects was restricted to cells expressing CCR-5. However, in contrast to previous reports [Björndal et al., 1997], only one of the five SI isolates characterized in this study showed a regular use of CXCR-4.

In summary, despite the strict definition of the long-term asymptomatic status applied in our study, the LTNP subjects were heterogeneous according to their virological status. However, they were significantly different from the asymptomatic slow progressor controls with regard to virus load, while no major difference in HIV replication properties and cell tropism were observed. Undetectable or very low viral plasma load also suggested that the viral replication was efficiently controlled over time in these subjects. The relative contributions of virological factors in determining the lack of progression of HIV disease still remain unclear in these subjects. During the first year follow-up, six LTNP subjects progressed regarding HIV-infection suggesting that long-term survival reflects a delay in the progression of the disease rather than a complete lack of progression, at least in some subjects [Soriano et al., 1997]. The hypothesis of a specific subgroup of LTNP patients, who will not develop disease cannot be ruled out as yet, since the follow-up indicated a great stability of virological parameters over this period for the majority of the LTNP subjects. Further follow-up studies are required to determine what percentage of LTNP subjects, meeting the clinical definition of long-term nonprogressors at entry, do not have progressive disease in the future.

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